## REMARKS/ARGUMENTS

PREMATURE FINALITY

Applicants respectfully traverse the imposition of finality in the Office Action mailed June 2, 2005. The basis of finality was alleged to be that the amendments to the claims "necessitated the new ground(s) of rejection presented". The new grounds resulted in the inclusion of rejections based on at least one or more of new documents by Zhang et al., Eberwine (WO 98/22624), Emmert-Buck et al., and Oku et al. None of these documents were previously relied upon.

Applicants respectfully point out, however, that there were only changes to claims 1, 2, and 4, none of which altered the scope of the claims or the features recited therein. More specifically, claim 1 was revised to expressly recite an inherent feature of the claim. The Office Action mailed June 2, 2005 provided no explanation of how such a revision introduced subject matter or concepts that would necessitate a new ground of rejection based on the above listed documents . The actual language of claim 1 after the revision is as follows, where the underlined text is that which was added.

> 1. A method for detecting the presence of a ligand in a cell or tissue sample, said method comprising,
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> contacting said sample with a binding agent, attached to a detectable nucleic acid molecule, which agent binds said ligand;
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> staining said sample to identify cells of interest;
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> capturing or isolating said cells of interest; and
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> detecting said nucleic acid molecule <u>in said captured or isolated cells of interest</u>,
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> wherein the presence of said nucleic acid molecule indicates the presence of said ligand.

As evident from the above, the act of "detecting said nucleic acid molecule" must refer back to the "detectable nucleic acid molecule" attached to the "binding agent" recited earlier in the claim. The "binding agent" is also referred to as having bound the "ligand in a cell or tissue sample". Thus the "binding agent", and so nucleic acid molecule, must be bound to the ligand in the cells of interest recited in the claim. Simply put, there was and is no other nucleic acid molecule to be detected. Accordingly, the recitation of "in said captured or isolated cells of interest" merely expressly recites a feature that was already present in claim 1 and so could not have created a basis for any new ground of rejection.

The changes to claims 2 and 4 were to simply re-write them in independent form from their previous status as dependent claims. Accordingly, the changes could not have created any new ground of rejection.

In light of the above, Applicants respectfully submit that the finality imposed in the Office Action mailed June 2, 2005 was premature. Accordingly, Applicants request that the finality be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 103(a)

Claims 1-7 and 19 were rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Zhang et al. and Emmert-Buck et al. Applicants have carefully reviewed the statement of the rejection as well as the cited references. Applicants respectfully traverse because no *prima facie* case of obviousness has been presented.

As an initial matter, Applicants again point out that the claimed methods require a number of features that are interrelated. Feature One is the act of contacting of a cell or tissue sample with a binding agent, attached to a detectable nucleic acid molecule, which agent binds a ligand in the sample. Feature Two is the act of staining the sample to identify cells of interest, which are captured or isolated. Feature Three is the act of detecting the detectable nucleic acid molecule in the captured or isolated cells to indicate the presence of the ligand. These features define methods that are different from those that are disclosed by a combination of Zhang et al. and Emmert-Buck et al., which is misplaced for the following reasons.

The statement of the rejection appears to acknowledge that Zhang et al. describe the detection of an antigen in a sample by use of an antibody binding agent attached to a nucleic acid molecule which is detected after the formation of an antigen/antibody complex. But this disclosure is quite different from the claimed methods, wherein Feature Two of staining and capturing cells after formation of a ligand/binding agent complex occurs **before** detection of a nucleic acid molecule attached to the binding agent. It is well settled that a *prima facie* case of obviousness against a claimed method requires motivation to modify a method of a reference to arrive at the claimed method. Thus a viable assertion of obviousness based on Zhang et al. must include a suggestion, reason, or motivation to modify the disclosure of Zhang et al. to include Feature Two.

But why would a skilled person interrupt the method of Zhang et al. to stain and capture cells before detection of the nucleic acid molecule? The statement of the rejection does not allege any suggestion or motivation from the teachings of Zhang et al. Moreover, Applicants respectfully submit that Zhang et al. actually provide no reason for an artisan of ordinary skill to stain and capture the cells because they specifically describe the use of melanoma cell lines and blood cells from healthy donors and melanoma patients. In the case of the cell lines, all cases showed antigen; and in the case of healthy donor cells, all cases showed no antigen (see page 1429 and Figure 1). Thus the use of the Zhang et al. method with such cells would have no need to stain or isolate any cell because they were essentially homogeneous. Zhang et al. also describe the detection in blood cells of melanoma patients as providing the ability to qualitatively detect the antigen as indicator of the presence of blood borne melanoma cells (see pages 1430-1, Figure 3, Table 1, and Discussion section). The methodology is also described as being "ultrasensitive" (see page 1428, left column, last sentence before Materials and Methods section). Given such a focus, why would an artisan of ordinary skill be motivated to introduce additional staining and capturing events which would not improve the qualitative assay disclosed by Zhang et al.? Applicants respectfully submit that the statement of the rejection fails to provide such motivation to do so.

Emmert-Buck et al. also fail to correct the lack of motivation for modifying Zhang et al. to arrive at the claimed methods. The statement of the rejection appears to

acknowledge that Emmert-Buck et al. describe the laser capture microdissection (LCM) of tissue sections by first staining the cells and then capturing select cells. But the statement of the rejection provides no reason why an artisan of ordinary skill would combine these teachings, regarding a tissue section of cells attached to other cells in the tissue architecture, with the cell lines or blood borne cells of Zhang et al. More specifically, why would one stain the melanoma or blood cells of Zhang et al., in a manner of Emmert-Buck et al., after they have been bound by the antibody in Zhang et al.'s method? And even if there was reason to perform such staining, where is the motivation to laser capture blood cells, which, unlike a tissue section, are not attached to each other?

The statement of the rejection also refers to the comment by Emmert-Buck et al. that PCR amplification of DNA or RNA from capture cells was not diminished. But how is this observation alone sufficient to provide a basis for using Emmert-Buck et al. to modify Zhang et al.? Applicants respectfully submit that it does not provide a basis for the modification because the amplification of DNA or RNA by Emmert-Buck et al. is with respect to the DNA or RNA endogenously present in the captured cells and not of any nucleic acid attached to a binding agent. Endogenously present DNA and RNA in captured cells are expected to be present. But the amount of a nucleic acid attached to a binding agent may vary greatly in amount from cell to cell, depending on the amount of binding agent associated with each cell. Thus the observation regarding PCR of endogenous nucleic acids cannot be directly related to the detection of nucleic acid molecules in the claimed methods.

The statement of the rejection included the following assertion of motivation:

> "[a]n ordinary practitioner would have been motivated to combine the method [] for detecting the presence of a ligand in a cell as taught by Zhang et al. with the step of staining and capturing cells of interest for the purpose of reducing the background noise of unwanted cells from heterogonous cells."

Applicants respectfully submit that the above assertion clearly fails to provide an adequate basis for the rejection. The assertion that "staining and capturing cells of interest for

the purpose of reducing the background noise of unwanted cells from heterogeneous cells" is inconsistent with the disclosure of Zhang et al. For example, page 1430 and Figure 3 in Zhang et al. describe the ability to detect melanoma cells even when they are diluted with non-melanoma PBLs (peripheral blood lymphocytes). Thus Zhang et al. actually added "unwanted cells" to the melanoma cells to be detected, which is the exact opposite of the above alleged motivation. And even with such an addition, the Zhang et al. method still worked. So why would there be motivation to remove "unwanted cells" from such a highly functional and sensitive method?

Moreover, where is problem of the "background noise of unwanted cells" in Zhang et al.? As noted above, Zhang et al.'s method worked even when additional "background" PBL cells were added. Additionally, the melanoma cell lines and healthy donor cells in Zhang et al. are homogeneous populations with respect to the antigen to be detected and so they are either all "wanted cells" or all "unwanted" cells which create no issue of "background noise." And while the blood cells from melanoma patients may contain cells without the antigen to be detected, *there is no teaching or suggestion of any stain that can be used to identify "unwanted cells"* in the blood cells of Zhang et al. Therefore, the above quote appears to be alleging that it would be obvious to do something (stain and capture cells from "unwanted cells") in the method of Zhang et al. where no means to differentiate "unwanted cells" from "wanted cells" is present. Applicants point out that the antibody of Zhang et al., which is alleged to be the binding agent in the claimed methods, cannot be used as the "stain" because the staining in the claimed methods is clearly not performed by the binding agent. The above quoted statement thus does not explain how the artisan of ordinary skill would arrive at the claimed methods via a combination of Zhang et al. and Emmert-Buck et al.

Given the above explanation, the instant allegation of obviousness appears to rely upon improper hindsight reconstruction of the invention using the instant application as a guide. This is especially evident given the assertion that separation of wanted and "unwanted" cells in Zhang et al. can be achieved in the absence of guidance from either Zhang et al. or Emmert-Buck et al.

In light of the foregoing, Applicants respectfully submit that no *prima facie* case of obviousness has been presented and this rejection may be properly withdrawn.

Claims 1-2, 6-7, 13-15, 18-19, and 21 were rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Eberwine (WO 98/22624) and Emmert-Buck et al. Applicants have carefully reviewed the statement of the rejection as well as the cited references. Applicants respectfully traverse because no *prima facie* case of obviousness has been presented.

The instant rejection is similar to the above addressed rejection based on Zhang et al. and Emmert-Buck et al. except that the disclosure of Eberwine is used in place of Zhang et al. But the teachings of Eberwine render the instant rejection even more misplaced.

Specifically, the portion of Eberwine relied upon in the statement of the rejection, page 6, lines 3-31, describe an "immuno-aRNA" method which includes use of "a first antibody ... immobilized to a solid support" (see lines 9-11) which is apparently used to immobilized cells prior to contact of the cells with "a second antibody ... which is covalently coupled to a RNA promoter-driven cDNA sequence so that the second antibody binds to bound selected protein on the solid support" (see lines 16-20). Therefore, the cells to be detected by the second antibody are already separated from other cells *by virtue of binding to the same ligand as bound by the second antibody* (see lines 9-10 for the description of the first antibody's specificity). So in the Eberwine method, only the cells that have the ligand are immobilized on the solid support and then detected.

But the instant statement of the rejection again relies upon the assertion of a need to reduce "background noise of unwanted cells" by staining and capturing. This is clearly not needed in the Eberwine method where "wanted" cells are already separated from "unwanted" cells by immobilization on the solid support. Why would an artisan of ordinary skill need to stain and capture cells from that solid support?

Applicants respectfully submit that no motivation to modify the Eberwine method to arrive at the claimed invention is provided. Emmert-Buck et al. certainly do not describe or suggest any need to stain and capture cells that have been immobilized by a first antibody (like that of Eberwine) which is directed to the very same ligand to be detected by a second antibody (as in Eberwine). In sharp contradiction to the instant statement of the rejection the artisan of

ordinary skill would recognize that Eberwine already immobilized the cells of interest and that no further staining or capturing or other isolation was necessary.

In light of the foregoing, Applicants respectfully submit that no *prima facie* case of obviousness has been presented and this rejection is misplaced. Withdrawal of the rejection is respectfully requested.

Claims 8-9 and 18 were rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Zhang et al. and Emmert-Buck et al. in view of Oku et al. Applicants have carefully reviewed the statement of the rejection as well as the cited references. Applicants respectfully traverse because no *prima facie* case of obviousness has been presented.

The failings of the Zhang et al. and Emmert-Buck et al. combination have been addressed above. Oku et al. is apparently relied upon solely for the disclosure regarding detection of a plurality of ligands. While Applicants no not acquiesce to any assertion as to the content of Oku et al., Applicants point out that Oku et al. do not correct any of the deficiencies noted above in the combination of Zhang et al. and Emmert-Buck et al. Therefore, this rejection must fail at least for the same reasons as that provided above for the failure of Zhang et al. and Emmert-Buck et al.

Accordingly, Applicants respectfully submit that no *prima facie* case of obviousness has been presented and this rejection should be withdrawn along with the above rejection based on Zhang et al. and Emmert-Buck et al.

Claim 20 was rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Eberwine (WO 98/22624) and Emmert-Buck et al. in view of Oku et al. Applicants have carefully reviewed the statement of the rejection as well as the cited references. Applicants respectfully traverse because no *prima facie* case of obviousness has been presented.

The failings of the Eberwine and Emmert-Buck et al. combination have been addressed above. Oku et al. is apparently relied upon solely for the disclosure regarding detection of a plurality of ligands by use of a microarray. While Applicants no not acquiesce to any assertion as to the content of Oku et al., Applicants point out that Oku et al. do not correct

any of the deficiencies noted above in the combination of Eberwine and Emmert-Buck et al. Therefore, this rejection must fail at least for the same reasons as that provided above for the failure of Eberwine and Emmert-Buck et al.

Accordingly, Applicants respectfully submit that no *prima facie* case of obviousness has been presented and this rejection should be withdrawn along with the above rejection based on Eberwine and Emmert-Buck et al.

Claims 1-3, 6-12 and 19 were rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Reiter et al. and Emmert-Buck et al. Applicants have carefully reviewed the statement of the rejection as well as the cited references. Applicants respectfully traverse because no *prima facie* case of obviousness has been presented.

As an initial matter, the disclosure of Reiter et al. relates to fluorescence *in situ* hybridization, or FISH. This is evident on pages 96 and 97 within the passages most heavily relied upon in the instant statement of the rejection. There are, however, at least two key distinctions between FISH and the claimed methods. First, and quite obviously, a FISH based method is conducted ***in situ***, which in Latin refers to "in the original context or place". Consistent with this meaning, Reiter et al. provide no teaching or suggestion of isolating cells from their original tissue architecture as found in a tissue sample. Stated differently, there is no teaching, suggestion, or other indication of capturing cells from a larger extracellular context in which the cells are found in a sample. Thus Reiter et al. would be considered to teach away from the concept of isolating cells from their *in situ* environment. This is in sharp contrast to the instant claims, which requires isolation or capture of cells.

Second, and because there is no isolation of cells, Reiter et al. fail to teach or suggest the staining of cells in a sample to facilitate their isolation or capture. The instant statement of the rejection refers to page 96, right column, second paragraph for the proposition that Reiter et al. teach the use of hematoxylin and eosin (H&E) staining. A careful review of that paragraph, however, reveals that of 15 tissue section slices for each case, only the first section of each 15 was so stained to "ascertain the region of interest". There is no teaching or suggestion that the same section was used for FISH, and certainly no teaching or suggestion of isolating or

capturing cells from that section. This failure to teach or suggestion cell isolation or capture remains even if, assuming *in arguendo*, the same stained section was used for FISH.

Thus Reiter et al. fail to disclose or suggest at least Features Two and Three as discussed above. Moreover, Reiter et al. would actually lead an artisan of ordinary skill away from the inclusion of Feature Two because it would remove the *in situ* nature of the Reiter et al. method.

The above deficiencies of Reiter et al. are not remedied by the disclosure of Emmert-Buck et al., which is alleged to disclose

> "a laser capture microdissection method for selective transfer and recovery of tissue samples using LCM, wherein Emmert-Buck et al. disclose tissue section fixed on glass slides are stained using histochemical staining (H & E stain) and the cells of interest were captured using laser capture microdissection ... before detecting the ligand by PCT".

But the above assertion clearly does not address in detail how the methods of Reiter et al. and Emmert-Buck et al. can be combined to arrive at Features One, Two and Three of the claims. While the instant rejection may be based on the argument that Emmert-Buck et al.'s process corresponds to Feature Two, where is the guidance or suggestion to perform the Emmert-Buck et al. process in the middle of Features One and Three, as allegedly present in Reiter et al., and result in the destruction of the *in situ* nature of the Reiter et al. method?

Simply put, the statement of the rejection provides no basis regarding *why* the artisan of ordinary skill would be willing to incur the loss of the information provided by *in situ* hybridization.

The instant statement of the rejection asserts that motivation is present

> "to detect the presence of a ligand in a manner taught by Emmert-Buck et al. using the capturing of cells of interest identified by staining said cells to achieve expected benefit of developing a sensitive method of detecting said ligand because Emmert-Buck et

> al. taught that the LCM provide selective capture of pure population of cells of interest from heterogeneous tissue, which minimizes contamination.... An ordinary practitioner would have been motivated to combine the method of for detecting the presence of a ligand in a cell as taught by Reiter et al. with the step of staining and capturing cells of interest for the purpose of reducing background noise of unwanted cells from heterogonous cells."

Applicants respectfully submit that the above is inadequate to meet the motivation required to support a *prima facie* case because 1) it attempts to combine Reiter et al.'s *in situ* based process with the Emmert-Buck et al. non-*in situ* process without providing motivation for *why* an artisan of ordinary skill would combine disparate processes; and 2) it fails to address why the cells in Reiter et al.'s method, which provide the *in situ* context relied upon in the method, would be considered "contamination".

Point 1) above is particularly important because Reiter et al.'s disclosure also describes the work as identifying cells that have increased copies of PSCA, MYC, or both nucleic acids. If Reiter et al.'s method were to be combined with that of Emmert-Buck et al. as asserted in the rejection, how would the ordinary artisan stain and capture only the cells that express PSCA, MYC, or both nucleic acids? As noted above, the use of hematoxylin and eosin (H&E) staining by Reiter et al. was not for the identification of cells with increased copies of PSCA, MYC, or both. Thus Emmert-Buck et al.'s use of H&E does not remedy the lack of guidance to the ordinary artisan.

Applicants respectfully point out that a careful consideration of Reiter et al. shows that the *in situ* context was important for separating out the cells which have increased copies of a) PSCA, b) MYC, or c) both because the *in situ* context provides the means to detect cells that fall into each of groups a, b, and c. If, as alleged in the statement of the rejection, all cells that might express PSCA, MYC or both were isolated from the *in situ* architecture, it would not be possible to identify which cell belonged to which group. This would destroy the Reiter et al.

method. If, assuming *in arguendo*, that the capturing of the cells could be made based on each of groups a, b, and c, then that would mean the staining and identification which precedes the capture would be sufficient to obviate the need for the Reiter et al. method of detecting the presence of each group. Therefore, and in both cases, the combination of Reiter et al. and Emmert-Buck et al., as alleged in the rejection, would lead to the destruction of the Reiter et al. method. As set forth at MPEP 2143.01, a proposed modification of disclosed subject matter cannot render the subject matter unsatisfactory for its intended purpose.

Point 2) above is also important because the instant statement of the rejection asserts that of the cells in the *in situ* material used by Reiter et al. would be considered to be "contamination". But as noted above, the power and purpose of the Reiter et al. method was to be able to detect cells that belong in at least three groups. Is the instant rejection alleging that some of these groups are "contamination"? If so, which one(s), and under what circumstances? Moreover, why would they be considered "contamination" when in the Reiter et al. method, their identification are the purpose of the method?

In light of the above, Applicants respectfully submit that there is a failure to present a *prima facie* case of obviousness based on the combination of Reiter et al. and Emmert-Buck et al. Accordingly, the instant rejection is misplaced and should be withdrawn.

Claims 16 and 17 were rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Reiter et al. and Emmert-Buck et al. in view of Wang et al. Applicants have carefully reviewed the statement of the rejection as well as the cited references. Applicants respectfully traverse because no *prima facie* case of obviousness has been presented.

The failings of the Reiter et al. and Emmert-Buck et al. combination have been addressed above. Wang et al. is apparently relied upon solely for the disclosure regarding use of a single antibody against AR to detect phosphorylated and unphosphorylated forms via Western Blotting (see pages 23-24, bridging paragraph). But a single antibody is not Thus a plurality of ligand binding agents as required by claims 16 and 17. Accordingly, this rejection fails to address all the requirements of the claims as required for a *prima facie* case.

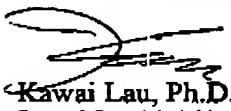
Moreover, and because Wang et al. fail to correct any of the deficiencies in the Reiter et al. and Emmert-Buck et al. combination as explained above, this rejection must fail at least for the same reasons as that provided above for the failure of Reiter et al. and Emmert-Buck et al. Accordingly, Applicants respectfully submit that this rejection should be withdrawn along with the above rejection based on Reiter et al. and Emmert-Buck et al.

## CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 858-350-6151.

Respectfully submitted,



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